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#### FIELD REPORT

## Effects of Stocking Densities on Growth of the Pacific White Shrimp (*Litopenaeus vannamei*) in Earthen Ponds

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#### Abstract

The effects of stocking density and physicochemical water characteristics on the growth, survival, and production of the Pacific white shrimp, *Litopenaeus vannamei*, were assessed in six 500±72.5 m<sup>2</sup> earthen ponds during two culture seasons: autumn-winter (20 weeks) and spring-summer (14 weeks). Stocking densities were 50, 60, and 70 shrimp/m<sup>2</sup>. Water turnover was approximately 15% per day. Aeration was supplied by a 3.5 hp air blower. Mean growth during the autumn-winter did not differ significantly among the stocking densities with final weights of 12.77, 12.72, and 12.40 g and yields of 3,609, 5,093, and 5,618 kg/ha at 50, 60, and 70 shrimp/m<sup>2</sup>, respectively. In contrast, there were statistically significant differences in mean growth and final yields during the spring-summer. Final mean weights were 18.63, 13.46, and 11.86 g and yields were 7,243, 7,307, and 8,011 kg/ha at 50, 60, and 70 shrimp/m<sup>2</sup>, respectively. Low water temperatures during the winter affected production. Better performance was recorded at higher spring-summer temperatures (≥23°C). Larger shrimp were associated with lower stocking density while higher stocking density produced slightly higher yields. For best performance, dissolved oxygen should be maintained above 4 mg/l.

#### Introduction

An increase in farmed shrimp production can be achieved by increasing stocking density but this requires an increase in feed input which may degrade water quality. The optimal stocking density varies depending on the farm

system and management practices. Stocking densities range 1-3 shrimp/m<sup>2</sup> in extensive, 10-50 shrimp/m<sup>2</sup> in semi-intensive, and up to 160 shrimp/m<sup>2</sup> in intensive farming systems. Stocking density is inversely proportional to

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shrimp growth. Therefore, production is optimized by using the appropriate stocking density for each farm.

In semi-intensive shrimp cultures, water exchange minimizes eutrophication without requiring additional aeration. Water exchange is required for intensive cultures to prevent phytoplankton blooms and agglomeration of toxic metabolites not assimilated by phytoplankton. Mechanical aeration (mainly at night) minimizes circadian changes of dissolved oxygen. A balanced food supplement improves growth and, together with aeration, is commonly used in intensive culture. As shrimp aquaculture technology advances, greater efficiency with respect to water conditions, survival, growth, and yield is required.

Farm yields have increased markedly due to technological advances. In semi-intensive *L. vannamei* culture, Lawrence et al. (1985) produced 1,825 kg/ha of 10 g shrimp in three months at a stocking density of 25 shrimp/m<sup>2</sup>. Clifford (1994) obtained 2,363 kg/ha of 18.5 g animals in 85 days with stocking densities of 18-22 shrimp/m<sup>2</sup>. Aragon and Garcia (1996) produced 2,743 and 4,442 kg/ha of 13.99 and 14.47 g shrimp in 120 days in stocking densities of 24 and 38 shrimp/m<sup>2</sup>, respectively. Manzo (2000) exceeded these values, producing 5,292 kg/ha in 130 days at a stocking density of 40 shrimp/m<sup>2</sup>. Martinez (1993) suggested that the optimal stocking density for a semi-intensive *L. vannamei* production system is 47.2 shrimp/m<sup>2</sup> for the autumn-winter cycle and 22 shrimp/m<sup>2</sup> for the spring-summer cycle.

Oxygen solubility in water is affected by temperature, salinity, organic matter content, and rate of photosynthesis (Zendejas, 1999). Dissolved oxygen concentrations of 0-1.5 mg/l are lethal for shrimp and concentrations of 1.7-3.0 mg/l result in low feed conversion and slow growth (Brock and Main, 1994). The optimum salinity for *L. vannamei* culture is 15-25‰ (Bray et al., 1994) and the optimal temperature for growth 27-30°C (Ponce et al., 1997). Other authors have suggested that temperature is a key factor affecting growth and production of shrimp (Teichert-Coddington et al., 1994; Jackson and Wang, 1998).

While much has been learned about the effects of individual variables on shrimp growth, studies have not yet integrated multiple variables into their experimental designs under intensive culture conditions. The aim of this study was to investigate the effects of stocking density and physicochemical water characteristics on the growth, survival, and production of the Pacific white shrimp (*L. vannamei*) during two culture seasons: autumn-winter and spring-summer.

### Materials and Methods

Two experiments were conducted at the El Naranjo campus of the University of Colima, Mexico. The experiments were conducted in six earthen ponds (500±73 m<sup>2</sup>) with an average water depth of 1.2 m. Post larvae (PL15) of *L. vannamei* from Acuagranjas del Pacifico Tecoman Col., Mexico, were stocked during two seasons: autumn-winter (20 weeks) and spring-summer (14 weeks). Ponds were stocked in duplicate at densities of 50, 60, and 70 shrimp/m<sup>2</sup>. Treatments were randomly assigned to ponds for each season.

Water was supplied from the Juluapan lagoon using a 6-inch pipe and a 15-hp engine at a rate of 50 l/s. Aeration was supplied overnight and on cloudy days with a 3.5-hp blower. Water temperature, dissolved oxygen (DO), and transparency were measured daily at 9:00 and 18:00. During the last two weeks of the summer season, OD concentrations in each pond were monitored hourly with a multiparameter CTD (YSI 6000).

After 30 days of culture, 15% of the water in each pond was exchanged daily for the remainder of the season. Shrimp were fed a commercial feed (Camaronina Agribands Purina®) with 40% protein during the first month and 35% thereafter. The feeding rate during the first week was 150% of the total biomass. It was reduced to 50% during the second week and to 10% during the third week. The feed ration was then adjusted to match the estimated changes in total biomass and reduced to 2% of the total biomass towards the end of the season.

Shrimp were sampled and weighed weekly to evaluate culture performance and adjust

feeding rates. The number of shrimp per sample was calculated according to Sparre and Venema (1998):

$$\varepsilon = \frac{t_{(n-1)} * \frac{\delta}{\bar{x}}}{\bar{x}} * 100$$

where  $\varepsilon$  = maximum relative error 10%,  $n$  = sample size,  $t_{(n-1)}$  = 95% confidence limits (student  $t$  distribution),  $\delta$  = standard deviation and  $\bar{x}$  = estimated mean.

Sixty specimens per pond were sampled each week by conducting five drags in different areas of the pond. Growth was calculated according to the formula: weight gained = (final weight-initial weight)/ $t$  (Sandifer et al., 1993). The feed conversion rate was estimated as: dry weight of feed/wet weight of shrimp (Bray et al., 1994). The survival rate was determined as:  $N_f/N_i \times 100$  (Cruz et al., 1999):

Bartlett and Kolmogorov-Smirnov tests were applied to test for data normality, independence, and homogeneity (Zar, 1994). A one-way ANOVA was used to determine differences among treatments in growth, production, and survival. Means were compared and ranked using Tukey's multiple range tests. A value of  $p \leq 0.05$  was considered significant (Daniel, 1999). A regression analysis (Cohen, 1988) was performed on temperature and DO.

### Results

The effects of stocking density on shrimp growth varied dramatically between seasons (Fig. 1). During the autumn-winter cycle, stocking density had no significant effect on shrimp growth. In the spring-summer cycle, however, shrimp growth differed significantly among densities (Table 1). The mean average survival for the autumn-winter cycle was 59.3%, significantly different from survival in spring-summer cycle (84.0%). There were significant differences in production among stocking densities in both seasons but the feed conversion ratio differed significantly only during autumn-winter.

Water quality parameters are shown in Table 2. Salinity was lowest in October-December (3‰) and gradually increased to 31.6‰. This parameter varied minimally dur-

ing the spring-summer period. The pH was similar throughout the season in both seasons. Dissolved oxygen levels were critically low during the last 15 days of spring-summer, especially in the 70 shrimp/m<sup>2</sup> treatment. At this stocking density, the oxygen concentration at the bottom of the pond dropped as low as 0.35 mg/l, despite the fact that the aeration system ran continuously. In response to the low oxygen concentrations, it was necessary to remove the sediment from the deepest part of the ponds, covering an area of 100 m<sup>2</sup> around the discharge zone, and to increase the daily water turnover to 60%.

The minimum DO concentrations were recorded at dawn (Fig. 2). Even at these times of low oxygen concentrations (e.g., 1.0 mg/l), the shrimp remained at the bottom of the ponds. The DO concentration occasionally dipped into the range of 0.35-0.68 mg/l and, then, the shrimp were observed at the water surface.

Regression analysis showed a significant correlation between water temperature and shrimp growth during autumn-winter ( $r = 0.7$  to  $0.85$ ; Fig. 3). In contrast, there was little to no correlation during spring-summer ( $r = 0.2$ ). Regression analysis of dissolved oxygen against shrimp growth showed no correlation in either season ( $r = 0.0011$  to  $0.18$ ).

### Discussion

Stocking density influences production on shrimp farms (Jackson and Wang, 1998). Our results showed no significant growth differences among stocking densities during the autumn-winter cultivation period. In contrast, significant differences were observed during the spring-summer, suggesting that water temperature is more important than stocking density when assessing shrimp growth. During autumn-winter, the weekly growth rate was 0.62-0.64 g/wk while during spring-summer, the average weight gains were 0.85-1.33 g/wk, similar to those reported by Sandifer et al. (1993) of 0.94 g/wk for 60 shrimp/m<sup>2</sup> and by Hopkins et al. (1993) of 1.0 g/wk at 44 shrimp/m<sup>2</sup>. Coman et al. (2002) found that temperature significantly affected growth of *P. japonicus* while Lucien-Brun (1989) noted that

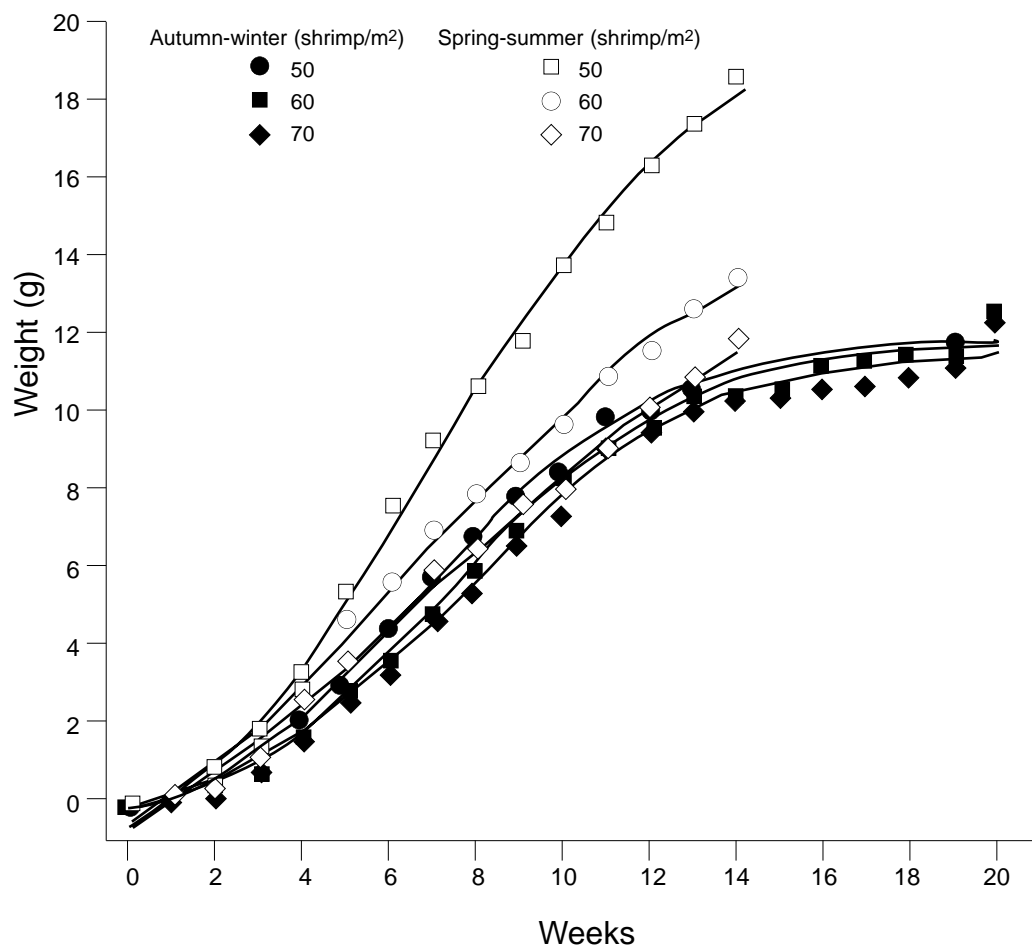


Fig. 1. White shrimp (*Litopenaeus vannamei*) growth curves in intensive culture conditions at different stocking densities during autumn-winter and spring-summer.

*L. vannamei* grew more slowly when the temperature was 22°C or below, with an optimal growth temperature of 23-34°C.

The average temperature during the spring-summer cycle was approximately 28°C, reported optimal for *L. vannamei* by Diaz et al. (2001). During this cycle the salinity remained around 33‰. Ponce et al. (1997) reported the optimum salinity for *L. vannamei* cultivated in Mexico is 33-40‰. According to

Diaz et al. (2001), *L. vannamei* experience the lowest level of stress at 27-30°C and salinities near the isosmotic point of 25-26.7‰.

Survival in this study is similar to that obtained by Sandifer et al. (1993) for *L. vannamei*, 69.5% at 60 shrimp/m<sup>2</sup>. For the same species, Hopkins et al. (1993) reported a survival rate of 81.9% at a stocking density of 44 shrimp/m<sup>2</sup> over the spring-summer period. During the same period, Escobedo (1994)

Table 1. Production of white shrimp (*Litopenaeus vannamei*) raised in different densities.

	Autumn-winter			Spring-summer		
Stocking density (shrimp/m <sup>2</sup> )	50	60	70	50	60	70
Surface area of pond (m <sup>2</sup> )	547.5	490	455	460	500	575
Final mean wt (g)	12.77 <sup>a</sup>	12.72 <sup>a</sup>	12.31 <sup>a</sup>	18.63 <sup>a</sup>	13.45 <sup>b</sup>	11.86 <sup>c</sup>
Duration (wk)	20	20	20	14	14	14
Survival (%)	55 <sup>a</sup>	65 <sup>a</sup>	57 <sup>a</sup>	75.91 <sup>a</sup>	85.9 <sup>b</sup>	90.44 <sup>c</sup>
Production (kg/ha)	3609 <sup>a</sup>	5093 <sup>b</sup>	5618 <sup>c</sup>	7243 <sup>a</sup>	7307 <sup>b</sup>	8011 <sup>c</sup>
Feed conversion ratio	1.94 <sup>a</sup>	2.08 <sup>b</sup>	2.07 <sup>c</sup>	1.04 <sup>a</sup>	1.01 <sup>a</sup>	0.96 <sup>a</sup>
Wt gain (g/wk)	0.63 <sup>a</sup>	0.64 <sup>a</sup>	0.62 <sup>a</sup>	1.33 <sup>a</sup>	0.97 <sup>b</sup>	0.85 <sup>c</sup>

Different letters within a growing season indicate significant differences ( $p < 0.05$ ).

reported a survival rate of 79.3% at 38 shrimp/m<sup>2</sup>. Despite the apparent consistency among these results, several factors were not detailed in these publications (e.g., temperature, DO concentration, feeding and fattening handling conditions, percent water turnover, feeding rate, pond bottom organic matter handling, aeration systems, etc.). Thus, further studies are required to determine optimal cultivation conditions with respect to stocking density, survival, yield, water quality management, and temperature.

The total yields obtained for the autumn-winter cycle were similar to those reported by Escobedo (1994), however, during the spring-summer cycle Escobedo (1994) reported a yield of 4,462 kg/ha at 38 shrimp/m<sup>2</sup> which differed markedly from values obtained in our study for the same period (7,243-8,011 kg/ha at 50-70 shrimp/m<sup>2</sup>). Our values also differ from those reported by Hopkins et al. (1993) of 5,718 kg/ha at 44 shrimp/m<sup>2</sup> and Sandifer et al. (1990) of 6,261 kg/ha at 60 shrimp/m<sup>2</sup>. Although direct comparison cannot be made between these studies, our results suggest that other variables in addition to the season and food quality, such as water quality, are important. Water characteristics, particularly temperature and DO content, appear to play key roles in achieving high yields. McGraw et

al. (2001) found that higher yields positively correlated with higher survival, as observed in our study.

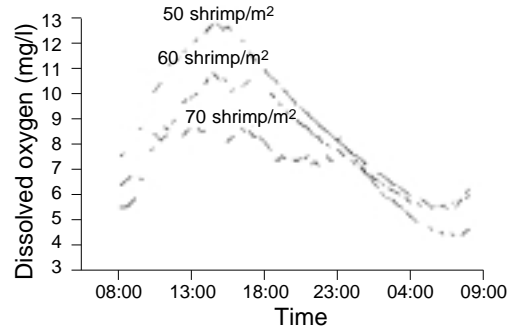
A remarkable feed conversion ratio (FCR) of 1:1 was recorded during the spring-summer cycle. Normally, values greater than 1.5-2.0:1 are obtained (Hopkins et al., 1993; Sandifer et al., 1990) and Samocha et al. (2004) reported values above 2.5:1. The difference may be attributed to environmental conditions and handling methods which favored primary productivity and elevated the natural food availability. The contribution of nutritional compounds originating from the natural food supply has been reviewed by Wouters et al. (2001).

During spring-summer, the lowest dissolved oxygen content was found in the highest stocking density and the highest oxygen concentration was found in the lowest. The elevated oxygen consumption in the high shrimp biomass suggests that the pond load capacity depends on water quality, particularly DO fluctuations. Villarreal (1984) reported that shrimp become stressed and their feeding rate drops at DO levels of 2 mg/l and below, resulting in depressed growth rates and poor food conversion ratio.

On the other hand, McGraw et al. (2001) concluded that shrimp growth is not affected

Table 2. Water quality parameters during pond culture of white shrimp (*Litopenaeus vannamei*); range, means $\pm$ SD.

	Autumn-winter (shrimp/m <sup>2</sup> )						Spring-summer (shrimp/m <sup>2</sup> )					
	50		60		70		50		60		70	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Transparency (cm)	16.0-45.0	25.8 $\pm$ 6.7	15.0-45.0	27.4 $\pm$ 7.3	13.0-45.0	28.3 $\pm$ 8.5	8.0-40.0	22.4 $\pm$ 7.2	10.0-40.0	23.5 $\pm$ 8.4	12.0-40.0	23.7 $\pm$ 9.9
Bottom temperature (°C)	23.1-31.3	26.7 $\pm$ 0.9	23.3-31.4	26.8 $\pm$ 0.96	22.6-30.5	26.8 $\pm$ 0.95	29.0-34.4	31.8 $\pm$ 0.8	27.5-35.9	31.80 $\pm$ 0.88	27.9-35.7	31.5 $\pm$ 0.94
Bottom DO 9:00 (mg/l)	0.5-13.5	4.9 $\pm$ 2.53	0.6-11.41	4.8 $\pm$ 2.16	0.7-9.4	3.7 $\pm$ 2.56	0.6-8.1	3.6 $\pm$ 1.82	1.4-8.9	4.37 $\pm$ 2.31	0.35-9.8	4.45 $\pm$ 1.43
Bottom DO 18:00 (mg/l)	3.6-19.6	10.9 $\pm$ 2.25	2.2-17.8	11.3 $\pm$ 2.03	1.2-18.0	9.7 $\pm$ 2.02	7.4-15.1	9.4 $\pm$ 1.78	5.7-16.3	10.90 $\pm$ 2.39	3.7-10.2	7.6 $\pm$ 1.62
Salinity (‰)	3.0-24.0	13.3 $\pm$ 1.4	3.5-25.0	13.8 $\pm$ 1.28	4.0-25.0	14.2 $\pm$ 1.35	31.7-34.4	33.2 $\pm$ 0.80	31.4-34.6	33.10 $\pm$ 0.92	31.4-34.9	33.5 $\pm$ 0.62
pH	7.8-9.0	8.5 $\pm$ 0.25	7.8-9.0	8.5 $\pm$ 0.28	7.4-8.9	8.3 $\pm$ 0.30	7.7-8.7	8.3 $\pm$ 0.03	6.9-8.7	8.30 $\pm$ 0.09	7.7-8.5	8.0 $\pm$ 0.13

Fig. 2. Average dissolved oxygen concentrations during a 24-hour cycle in intensively farmed shrimp (*Litopenaeus vannamei*) culture, late in the spring-summer season.

by low oxygen concentrations and Seidman and Lawrence (1985) reported the critical DO point for shrimp as being below 2 mg/l. Aragon and Garcia (1996) recommend that the minimum oxygen concentration for optimal shrimp growth be approximately 5 mg/l. In our study, the average oxygen concentration for each treatment was above the range considered stress inducing (approximately 3.0 mg/l; Brock and Main, 1994).

There were some critical moments in our study concerning dissolved oxygen. At dawn during spring-summer, a concentration of 0.35 mg/l was recorded. When concentrations below 1.0 mg/l were measured, shrimp were observed swimming at the surface. A similar observation was reported by Hopkins et al. (1993) who attributed migration to the surface to total suspended solids and the biochemical oxygen demand (BOD) at the bottom of the pond. During spring-summer, the temperature reached 37.5°C. Boyd (2001) reports that temperatures above 35°C result in reduced DO concentrations. Stress may be induced by the greater rate of excretion of ammonia-N under these conditions (Dong-Huo et al., 2000). Together, these factors negatively affect the general behavior, survival, and growth of penaeid shrimp (Ocampo et al., 2000; Coman et al., 2002).

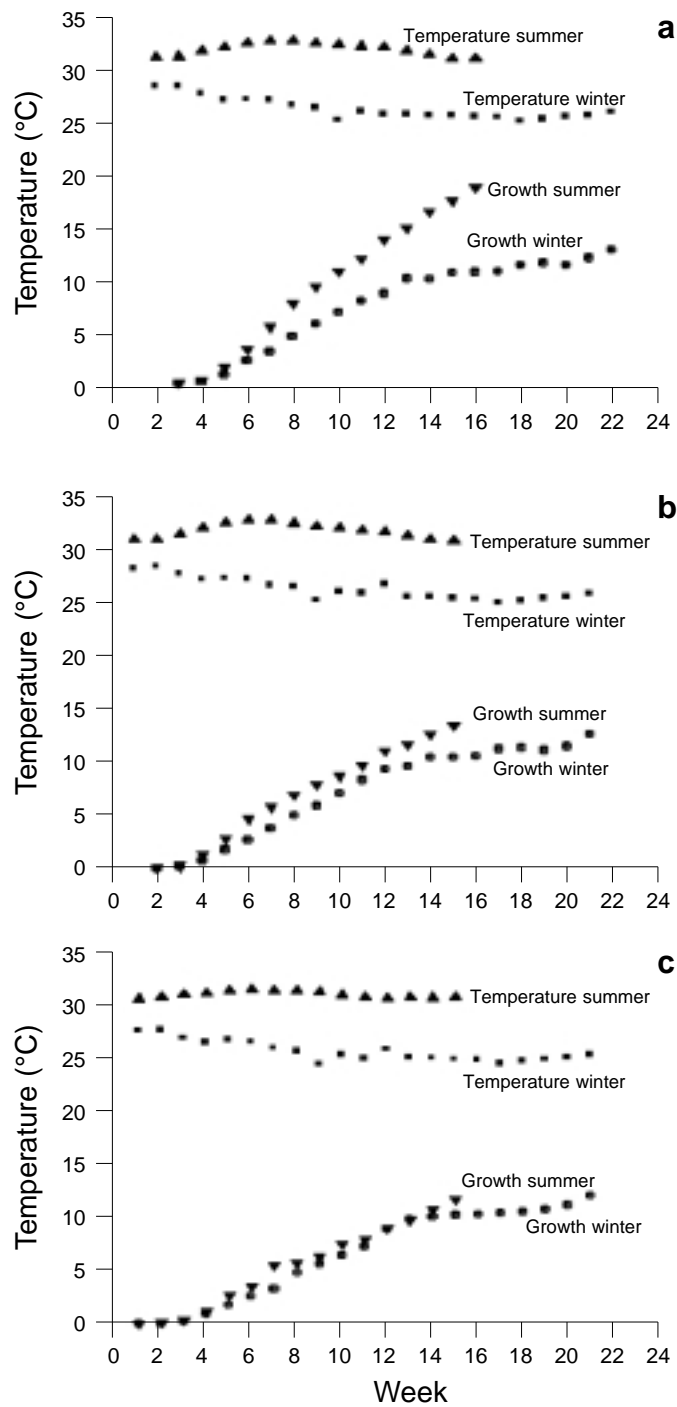


Fig. 3. Average water temperatures and correlation with growth in intensive culture of white shrimp (*Litopenaeus vannamei*) at stocking densities of (a) 50, (b) 60, and (c) 70 shrimp/m<sup>2</sup>.



In terms of shrimp size, better performance was associated with lower stocking densities. In terms of yield, slightly higher yields were associated with higher stocking densities. In general, we suggest culturing shrimp at high densities when temperatures are low followed by fattening to market size at lower densities when temperatures are more favorable. For best performance, dissolved oxygen should be maintained above 4 mg/l.

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